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Traditional risk factors for *Helicobacter pylori* infection not found among patients undergoing diagnostic upper endoscopy—Republic of Georgia, 2007–2008

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SUMMARY

Objectives: *Helicobacter pylori* causes gastritis, duodenal ulcers, and gastric cancer. Although household crowding, low socioeconomic status (SES), and poor sanitation are associated with infection elsewhere, risk factors of infection in the Republic of Georgia (ROG), a country with a high prevalence rate (>70%), remain unknown. In this study we explored potential risk factors of infection among symptomatic patients in ROG.

Methods: During 2007–2008, we prospectively recruited 390 subjects with gastrointestinal symptoms referred to five tertiary care centers for diagnostic upper endoscopy. We administered a questionnaire on potential risk factors and tested patients using three diagnostic tests: gastric biopsies underwent histological evaluation and rapid urease test (CLO test), and an ELISA was used to detect IgG against *H. pylori* in serum. We defined a case as having two or more positive results from the three available tests. Univariate and multivariate logistic regression analyses were performed.

Results: Overall, 217 (56%) patients met the study case definition. Subjects diagnosed with cancer had the highest rate of *H. pylori* infection (62%), followed by those with gastritis (55%), and ulcer (54%). Age >30 years (adjusted odds ratio (aOR 2.6, 95% confidence interval (CI) 1.6–4.3) and residing in the capital city (aOR 0.6, 95% CI 0.4–0.9) were significantly associated with infection.

Conclusions: In this large cohort with gastrointestinal symptoms, only age >30 years and living in the capital were significant factors associated with infection. Lower SES, less education, and crowding did not confer an increased risk, in contrast to the findings of previous studies. Population-based studies are needed to identify potential routes and risk factors of *H. pylori* infection in ROG.

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1. Introduction

The bacterium *Helicobacter pylori* has been implicated as a cause of gastritis, peptic ulcers, and gastric malignancies.[1–3] *H. pylori* infection occurs worldwide and is one of the most common bacterial infections among humans; an estimated 50% of the world's population is infected,[4] but only 10–20% of infected persons become symptomatic.[5] *H. pylori* infection is typically acquired in early childhood and usually persists throughout life unless specific treatment is applied.[6] Routes of transmission have not been well characterized; person-to-person transmission via fecal–oral, oral–oral, and gastric–oral routes have been proposed. Many studies also indicate low socioeconomic status (SES), including domestic crowding during childhood, as a major risk factor for higher infection prevalence.[7,8] Prevalence is higher and infection occurs earlier in life in developing than in developed countries.[9] The accuracy of the various diagnostic methods has been estimated by comparison with different standards. No unanimity exists about which method represents an appropriate gold standard.[10,11]

Limited information exists on *H. pylori* epidemiology in the Caucasus region. In the Republic of Georgia (ROG), a Caucasus region post-Soviet country with an economy in transition, more than 70% of adults are infected with *H. pylori*[12,13] and the reported prevalence of gastric cancer is 18 cases per 100 000 population,

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approximately 6- to 9-fold higher than in the USA.[14] We previously reported that 72% of the patients undergoing upper endoscopy in Georgia were histologically positive for *H. pylori*;[14] the retrospective nature of that study precluded analysis of relationships between demographic characteristics and infection. The present study was performed to elucidate potential risk factors of *H. pylori* infection among symptomatic patients in ROG, and to explore clinical and pathologic features of persons infected with *H. pylori*.

Understanding the epidemiology and risk factors of *H. pylori* infection is essential to better characterize disease associated with this pathogen and to design targeted, cost-effective prevention strategies.

2. Materials and methods

We conducted a study among 390 adults and children who sought care from adult and pediatric gastroenterologists and underwent diagnostic endoscopy in accordance with the clinical judgment of the attending gastroenterologist. Potential subjects were recruited consecutively from five tertiary healthcare centers of Georgia located in two large cities (Tbilisi and Marneuli) during July 2007–November 2008. The study was approved by the Institutional Review Board of the National Center for Disease Control and Public Health (NCDC) of Georgia (Tbilisi, ROG). A standard questionnaire was administered to all participants and to parents of children aged <16 years. Participants were asked about demographic characteristics, potential behavioral risk factors, and clinical symptoms experienced during the preceding 30 days.

Sociodemographic characteristics included sex, age group (aged >30 or ≤30 years), educational level attained (university or less than university level), area of residence (residing in rural or urban area), residence in the capital city Tbilisi, household income (≤600 Georgian Lari (GEL) per month; 1 GEL = US$0.6), and household crowding (>1.5 persons/room).

Potential behavioral characteristics included ever smoking, alcohol (any type) consumption, sources of drinking water (not mutually exclusive), boiling drinking water, current contact with any animal (either farm animal or pet), any medications taken 2 weeks before endoscopy, having household members with symptoms similar to the patient, and having first- or second-degree relatives diagnosed with gastritis, ulcer, or cancer.

Clinical symptoms experienced during the preceding 30 days included: heartburn, abdominal pain after eating food, abdominal pain relieved by eating food, abdominal pain in the night, nausea and vomiting, burping, hematemesis, weight loss, constipation, diarrhea, bad breath, early satiety, and melena.

2.1. Case definition

To confirm *H. pylori* infection in a study subject, we used three diagnostic tests: histological presence of the bacteria in biopsy samples taken via endoscopy (grades ≥1),[15] rapid urease test (CLO test; Campylobacter-like organism test) performed on a biopsy sample, and ELISA testing to detect IgG against *H. pylori* in serum. Any subject with two or more positive tests was defined as infected with *H. pylori*.

2.2. Histological diagnosis

One biopsy specimen from either the antrum or the corpus of the stomach was obtained from each study patient during diagnostic endoscopy. Two pathologists reviewed hematoxylin and eosin (H&E)-stained slides prepared from formalin-fixed, paraffin-embedded gastric biopsy specimens. If *H. pylori* was not detected in H&E-stained slides, Warthin–Starry stain was employed to increase the diagnostic sensitivity. The pathologists detected and graded the amounts of acute and chronic inflammation, intestinal metaplasia, and atrophy according to the visual analogue scale of the updated Sydney classification system for gastritis.[15] For study purposes we dichotomized histological characteristics as either presence (grades ≥2) or absence (grades ≤1) of each feature.

2.3. Rapid urease (CLO) test

During the diagnostic endoscopy, the study endoscopist performed a rapid urease test (CLO test; Ballard Medical Products, Draper, Utah, USA) on fresh biopsy specimens following the manufacturer's instructions.

2.4. ELISA

Serum specimens obtained by venipuncture were separated and transported on ice to the NCDC of Georgia (Tbilisi, ROG). Serum samples were tested for the presence of anti-*H. pylori* IgG antibody using a well-validated research ELISA.[16,17] Serum samples (10 μl) were tested by means of a standard 96-well microtiter plate ELISA spectrophotometer (Bio-Tek Synergy HT reader) at a wavelength of 492 nm. Specimens with an optical density (OD) of 0.86 were considered negative, while those with an OD of 1.32 were considered positive for the presence of anti-*H. pylori* antibodies. Values in between were designated as indeterminate. In addition to the initial serologic testing, 160 patients were retested during follow-up visits. During the analysis, indeterminate ELISA results were reclassified as negative because 68% of patients with an indeterminate result during the initial visit tested negative at the follow-up visit.

2.5. Statistical analysis

Data analysis was conducted using SAS (version 9.2; SAS Institute, Inc., Cary, NC, USA). A univariate analysis was conducted to explore how each of the subject, clinical, and histological characteristics is associated with *H. pylori* infection. Statistical significance was determined using Chi-square tests and was defined as a p-value of <0.05.

In multivariable logistic regression, we included variables that were significant in univariate analysis and those believed a priori to be associated with infection. Potential risk factors independently associated with a positive test result for infection were investigated by binary logistic regression using SAS, with adjustment for age group, residence, sex, household members with similar symptoms, household crowding, education, and any medication taken 2 weeks prior to endoscopy. Statistical significance was defined as a p-value of <0.05.

3. Results

We recruited 390 subjects from five tertiary healthcare centers serving all of ROG. All eligible subjects who were approached agreed to participate in the study. Study subjects resided in 11 of 13 regions of the country; 66% resided in the capital, Tbilisi. Seventy-seven percent of study subjects were evaluated at The Center of Endoscopy located in Tbilisi. The median age of study subjects was 43 years (range 2–81 years); 210 (54%) were female. *H. pylori* was detected in 269 (69%) subjects by histology, 196 (50%) by rapid urease test, and 183 (47%) by serology. Two hundred seventeen (56%) subjects met the case definition for *H. pylori* infection (Table 1).

3.1. Univariate analysis

Participants who were members of households that use spring water as one of their sources of drinking water were 2.9 (95%

Table 1
Helicobacter pylori diagnostic test results, alone and in combination—Republic of Georgia, 2007–2008

Diagnostic test	Number positive (%)
Histology	269 (69%)
Rapid urease test[a]	196 (51%)
ELISA	183 (48%)
Positive results ≥2 tests	217 (56%)
Positive results by histology, rapid urease test,[a] and ELISA	97 (25%)
Positive results by histology and rapid urease test[a]	143 (37%)
Positive results by histology and ELISA	135 (35%)
Postive results by rapid urease test[a] and ELISA	133 (34%)

[a] CLO test.

confidence interval (CI) 1.3–6.3) times more likely to receive a diagnosis of *H. pylori* than members of households who did not have spring water as a source of water. Odds were also higher for those who had contact with any type of animal (odds ratio (OR) 1.6, 95% CI 1.1–2.4) (Table 2). Living in the capital city Tbilisi was protective (OR 0.6, 95% CI 0.4–0.9).

The following risk factors were not significantly associated with *H. pylori* infection: education less than university level, self-reported household income of ≤600 GEL per month (the poverty level cutoff), household crowding (defined as >1.5 persons per room), consumption of alcohol (any amount) or tobacco, and frequency of alcohol consumption (Table 2). No single clinical sign or symptom was significantly associated with *H. pylori* infection (Table 3).

3.2. Histology

Overall, 303 (78%) antral biopsies and 85 corpus (22%) biopsies were obtained; the site was not specified for two biopsies. *H. pylori* was visualized on H&E-stained slides in 232 (77%) of the antral biopsies and 22 (26%) of the corpus biopsies. Warthin–Starry stain revealed the presence of bacteria in 15 (11%) of 136 biopsies in which the H&E-stained slides were negative for the organism. Biopsies in which *H. pylori* was detected were obtained from 181 (67%) subjects who met the case definition and 88 (33%) who did not; biopsies in which *H. pylori* was not detected were obtained from 85 (70%) subjects who did not meet the case definition and 36 (30%) who did.

On univariate analysis, the odds of having acute inflammation (OR 2.5, 95% CI 1.6–3.8) and having lymphoid nodules (OR 2.8, 95% CI 1.5–5.3) were significantly higher in biopsies that showed *H. pylori* infection (Table 4). Histologically, 286 (73%) study subjects were diagnosed with gastritis, 57 (15%) with peptic ulcer, and 47 (12%) with cancer (not presented in the table). *H. pylori* was detected in biopsies from 62% of subjects with cancer, 55% of those with gastritis, and 54% of those with ulcer.

3.3. Multivariate analysis

The final multivariable model included age, residence, sex, household members with similar symptoms, household crowding, education, and any medication taken 2 weeks prior to endoscopy. Only age >30 years (adjusted OR 2.6, 95% CI 1.6–4.3) and residing in the capital (aOR 0.6, 95% CI 0.4–0.9) were significantly associated with infection (Table 5).

Table 2
Characteristics of patients and risk factors for *Helicobacter pylori* infection, univariate analysis—Republic of Georgia, 2007–2008

Characteristics and exposures of study patients	*H. pylori*-positive (n = 217), n (%)	*H. pylori*-negative (n = 173), n (%)	OR	95% CI
Age (years)				
>30	179 (61)	113 (39)	2.5	1.6–4.0
≤30	38 (39)	60 (61)		
Sex				
Male	95 (53)	85 (47)	0.8	0.5–1.2
Female	122 (58)	88 (42)		
Residence				
Urban	168 (53)	148 (47)	0.6	0.3–1.0
Rural	48 (66)	25 (34)		
Residence in capital city, Tbilisi				
Tbilisi	131 (51)	126 (49)	0.6	0.4–0.9
Outside of Tbilisi	86 (65)	47 (35)		
Household income				
≤600 GEL	138 (57)	106 (43)	1.1	0.7–1.8
>600 GEL	59 (53)	52 (47)		
Education				
Less than university degree	99 (58)	73 (42)	1.1	0.8–1.7
University	118 (54)	100 (46)		
Ever smoked				
Yes	86 (53)	77 (47)	0.8	0.5–1.2
No	131 (58)	96 (42)		
Alcohol consumption (any)				
Yes	135 (56)	107 (44)	1.0	0.7–1.5
No	82 (55)	66 (45)		
Sources of drinking water (not mutually exclusive)				
Tap				
Yes	169 (54)	145 (46)	0.7	0.4–1.1
No	48 (63)	28 (37)		
Well				
Yes	26 (63)	15 (37)	1.4	0.7–2.8
No	191 (55)	158 (45)		
Bottled water				
Yes	59 (53)	53 (47)	0.8	0.5–1.3
No	158 (57)	120 (43)		
River				
Yes	3 (60)	2 (40)	1.2	0.2–7.3
No	214 (56)	171 (44)		

Table 2 (*Continued*)

Characteristics and exposures of study patients	*H. pylori*-positive ($n = 217$), n (%)	*H. pylori*-negative ($n = 173$), n (%)	OR	95% CI
Spring				
Yes	30 (77)	9 (23)	2.9	1.3–6.3
No	187 (53)	164 (47)		
Boil drinking water				
Yes	7 (50)	7 (50)	0.8	0.3–2.3
No	209 (56)	166 (44)		
Household crowding (>1.5 persons/room)				
Yes	45 (54)	39 (46)	0.9	0.6–1.5
No	172 (56)	134 (44)		
Child (aged <16 years) ever breastfed				
Yes	10 (48)	11 (52)	8.2	0.4–171.6
No	0	4 (100)		
Current contact with animals (either farm animal or pet)				
Yes	94 (63)	56 (37)	1.6	1.1–2.4
No	123 (51)	117 (49)		
Household member with similar symptoms				
Yes	49 (61)	31 (39)	1.3	0.8–2.2
No	168 (55)	140 (45)		
Household member diagnosed with gastritis, ulcer, or cancer				
Yes	32 (62)	20 (38)	1.0	0.4–2.5
No	19 (61)	12 (39)		
First- or second-degree relative diagnosed with gastritis, ulcer, or cancer by a physician				
Yes	77 (55)	64 (45)	0.9	0.6–1.4
No	125 (57)	93 (43)		
Any medication taken 2 weeks before endoscopy				
Yes	104 (54)	87 (46)	0.9	0.6–1.4
No	113 (57)	86 (43)		
Proton pump inhibitors				
Yes	54 (59)	37 (41)	1.2	0.8–2.0
No	163 (55)	136 (45)		
H_2 blockers				
Yes	6 (35)	11 (65)	0.4	0.2–1.2
No	211 (57)	162 (43)		
Non-steroidal anti-inflammatory agents				
Yes	22 (65)	12 (35)	1.5	0.7–3.2
No	195 (55)	161 (45)		
Any antimicrobial				
Yes	22 (48)	24 (52)	0.7	0.4–1.3
No	195 (57)	149 (43)		

OR, odds ratio; 95% CI, 95% confidence interval.

4. Discussion

To our knowledge this is the largest study examining the risk factors and prevalence of *H. pylori* infection among symptomatic patients undergoing diagnostic upper endoscopy in ROG.

Diagnostic techniques for *H. pylori* detection fall into two major categories: biopsy-based (invasive) tests including histological examination, CLO test, and culture or PCR of biopsy samples, and non-invasive tests such as ELISA (detection of *H. pylori* antibodies in serum or urine, detection of *H. pylori* antigens in stool) and carbon isotope breath tests.[10,11] Any of these tests may produce false-negative results under certain circumstances. The density and anatomical distribution of *H. pylori* colonization varies over time in achlorohydric patients, including those treated with acid inhibitors, resulting in false-negative results of either the rapid urease test, histological examination, or primary culture. In addition, sampling errors during endoscopy may result in the collection of organism-free biopsy tissue specimens from an infected patient. Thus, in clinical settings in which a high prevalence of *H. pylori*- related pathology is expected, any one biopsy-based method may not be sufficient to reliably detect infection. In such instances, the use of additional confirmatory methods to identify true cases of *H. pylori* infection is recommended.[18] Accordingly, we employed three diagnostic methods for the definitive diagnosis of *H. pylori* in our study. The low positivity of the ELISA and rapid urease test compared with histology rendered our study case definition (two or more positive results from the three available tests) restrictive, and may have resulted in an under-measurement of *H. pylori* prevalence in subjects. If histological diagnosis alone were used, the prevalence would have been 69% instead of 56%. The well-validated ELISA assay we used has shown a sensitivity of 92% and specificity of 98% in many other populations.[16,17] We note that in another study conducted in ROG,[19] this ELISA had a sensitivity of 76% compared with the urea breath test (UBT). The performance of the ELISA in ROG may be due to some unknown population-specific host factor or to the prevalent *H. pylori* strains. In the clinical setting, histology or UBT should be used in ROG for *H. pylori* diagnosis. Histological diagnosis has the added advantage of defining pathologies such as ulcers or the presence of an ulcerated cancer.

Our study demonstrated several distinct epidemiological features of *H. pylori* infection in the ROG population we studied. The overall prevalence of *H. pylori* infection was 56%, similar to that in studies elsewhere that have employed similar diagnostic modalities in symptomatic patients.[20] Unlike previous studies elsewhere,[21] less education, household crowding, and low SES were not associated with infection. The Georgian population is highly educated, with a large proportion of university and graduate degree holders (44% of our study subjects), which might indicate that risk factors other than those related to poor education play a larger role in the country. It is possible that the particular circumstances in Georgia at this time account for the discrepancy with studies elsewhere. The economic collapse accompanying the dissolution of the Soviet Union precipitously affected all levels of society, so that a person's current SES may be lower than that at birth.

Socioeconomic conditions in Georgia deteriorated over the last 20 years, following the break-up of the Soviet Union and a period of

Table 3
Signs and symptoms among patients evaluated by diagnostic endoscopy, univariate analysis—Republic of Georgia, 2007–2008

Clinical signs and symptoms	*H. pylori* positive ($n=217$), *n* (%)	*H. pylori* negative ($n=173$), *n* (%)	OR	95% CI
Heartburn				
Yes	131 (59)	91 (41)	1.4	0.9–2.1
No	86 (51)	82 (49)		
Abdominal pain after eating food				
Yes	117 (56)	92 (44)	1.0	0.7–1.5
No	99 (55)	80 (45)		
Abdominal pain relieved by eating food				
Yes	89 (56)	69 (44)	1.0	0.7–1.6
No	127 (55)	103 (45)		
Abdominal pain in the night				
Yes	76 (59)	53 (41)	1.2	0.8–1.9
No	141 (54)	120 (46)		
Nausea and vomiting				
Yes	108 (59)	76 (41)	1.3	0.8–1.9
No	109 (53)	97 (47)		
Burping				
Yes	125 (56)	100 (44)	1.0	0.7–1.5
No	91 (55)	73 (45)		
Hematemesis				
Yes	19 (56)	15 (44)	1.0	0.5–2.1
No	198 (56)	158 (44)		
Weight loss				
Yes	86 (59)	61 (41)	1.2	0.8–1.8
No	130 (54)	109 (46)		
Constipation				
Yes	88 (61)	57 (39)	1.4	0.9–2.1
No	129 (53)	116 (47)		
Diarrhea				
Yes	26 (48)	28 (52)	0.7	0.4–1.3
No	191 (57)	145 (43)		
Bad breath				
Yes	85 (57)	65 (43)	1.0	0.7–1.6
No	131 (56)	103 (44)		
Early satiety				
Yes	46 (55)	37 (45)	1.0	0.6–1.6
No	171 (56)	136 (44)		
Melena				
Yes	22 (55)	18 (45)	1.0	0.5–1.9
No	194 (56)	153 (44)		

OR, odds ratio; 95% CI, 95% confidence interval.

Table 4
Selected histological characteristics of biopsies from patients evaluated by diagnostic endoscopy, univariate analysis—Republic of Georgia, 2007–2008

Selected histological characteristics	*H. pylori* positive ($n=217$), *n* (%)	*H. pylori* negative ($n=173$), *n* (%)	OR	95% CI
Acute gastritis (presence of neutrophils)				
Yes	111 (69)	51 (31)	2.5	1.6–3.8
No	106 (46)	122 (54)		
Chronic gastritis (excessive mononuclear inflammatory cells)				
Yes	168 (59)	117 (41)	1.6	1.0–2.6
No	49 (47)	56 (53)		
Atrophy				
Yes	66 (60)	44 (40)	1.3	0.8–2.0
No	151 (54)	129 (46)		
Lymphoid nodules				
Yes	43 (75)	14 (25)	2.8	1.5–5.3
No	174 (52)	159 (48)		
Incomplete intestinal metaplasia				
Yes	55 (50)	55 (50)	0.7	0.5–1.1
No	162 (58)	118 (42)		
Complete intestinal metaplasia				
Yes	20 (45)	24 (55)	0.6	0.3–1.2
No	196 (57)	147 (43)		
Erosions				
Yes	216 (56)	172 (44)	1.3	0.1–20.2
No	1 (50)	1 (50)		

OR, odds ratio; 95% CI, 95% confidence interval.

Table 5
Multivariable logistic regression analysis final model: characteristics of patients and risk factors for *H. pylori* infection—Republic of Georgia, 2007–2008

Predictor of case status	Adjusted OR	95% CI
Age >30 years	2.6	1.6–4.3
Residence in capital city, Tbilisi	0.6	0.4–0.9
Male sex	0.8	0.5–1.3
Household member with similar symptoms	1.4	0.8–2.4
Household crowding (>1.5 persons/room)	1.1	0.7–1.9
Education less than university graduate	1.1	0.7–1.8
Any medication taken 2 weeks before endoscopy	0.9	0.6–1.5

OR, odds ratio; 95% CI, 95% confidence interval.

internal conflicts. Given higher living standards before 1991, and the general tendency to acquire *H. pylori* infection during childhood, a lower prevalence of infection might have been expected among older patients (similar to findings in industrialized nations) than among patients aged <30 years.

Overall, younger age and residence in Tbilisi were highly protective in the multivariate model. Tbilisi, the capital of Georgia, with a population >1 million, is the largest and the most developed city in the country. Sixty-six percent of study patients were residents of Tbilisi. In general, patients residing in Tbilisi were significantly younger than non-Tbilisi residents. For subjects residing outside Tbilisi, age was not significantly associated with infection, while for residents of Tbilisi, age was significantly associated with infection. Infected Tbilisi residents were significantly older than Tbilisi residents testing negative. That may be because we documented residence at the time of enrollment, leaving us unable to account for residence outside the capital earlier in life.

Contact with animals was associated with *H. pylori* infection in the univariate analysis. To date, humans have been reported to be infected by at least 11 different *Helicobacter* species, most of which are commonly or potentially pathogenic, and are likely zoonotic and transmitted from companion or farm animals.[21,22] *H. pylori*, however, is not known to infect non-primates.[23] Therefore, contact with pets may be a marker for another risk factor; alternatively, our exposed study subjects may have been infected with non-pylori *Helicobacter* species as well.[24] Diagnostic tests employed in the current study cannot distinguish *H. pylori* from other *Helicobacter* species.

Previous studies have demonstrated controversial and conflicting findings regarding signs and symptoms.[25] In our study there was no difference in the signs and symptoms of infected and non-infected subjects. No statistically significant difference was detected after stratifying characteristics by histological diagnosis (data not shown). This finding suggests that diagnostic testing is indicated before initiating treatment of *H. pylori* infection.

Previous studies have reported a high frequency of gastrointestinal symptoms, precancerous lesions, and *H. pylori* infection among relatives of *H. pylori*-infected patients,[26] and several authors have proposed routine screening and eradication therapy for persons with a family history of gastric cancer,[27] or for the general population in high risk areas.[28] Our study also showed a high frequency of gastrointestinal symptoms and *H. pylori*-associated conditions among relatives and household members of infected and uninfected subjects, suggesting a possible benefit to evaluating the close contacts of symptomatic patients in ROG.

Histological findings were consistent with previous studies. *H. pylori* infection was strongly associated with acute and chronic

inflammation but not in areas of intestinal metaplasia. *H. pylori* requires gastric mucus for growth, and mucus produced by the metaplastic and neoplastic cells is postulated to lack characteristics that sustain the growth of *H. pylori*.[28] When *H. pylori* has been observed in patients with ulcers, intestinal metaplasia, and adenocarcinoma, the bacteria are usually present in areas of the stomach not exhibiting these lesions.[29]

Our study had several limitations. First, the biopsy procedure involved substantially fewer biopsies per patient than is recommended; study subjects refused biopsy several times and were therefore biopsied from either the antrum or corpus; none were biopsied from both sites. In 78% of cases one antral biopsy specimen was obtained and in 22% of cases one corpus biopsy, instead of the five antral and corpus biopsy specimens recommended for diagnosis by the updated Sydney classification system.[15] Second, a substantial proportion (49%) of study subjects had taken medications 2 weeks before endoscopy. These two limitations might have resulted in under-diagnosis of infection due to under-detection or suppression of the organism.[18,30] Third, data regarding risk factors of infection should be interpreted with caution because our study population consisted of symptomatic subjects who presented for care, and not the general population. Fourth, socioeconomic and behavioral risk factors were self-reported by study patients.

Potential misclassification when using multiple combinations of diagnostic test results in the case definition can underestimate the true prevalence of infection and limit the study's ability to identify risk factors. The fact that ELISA and CLO detected fewer cases, substantially lowering the high prevalence detected by histology alone, could be explained by some unknown population-specific host factor or to the prevalent *H. pylori* strains in this part of the world. This indicates the need for more research to determine the optimal combination of tests for diagnosis.

In conclusion, we have shown that over half of symptomatic persons referred for upper endoscopy in ROG have a condition attributable to active *H. pylori* infection. Several risk factors related to SES in other populations do not appear to hold in ROG, highlighting the potential variability in risk factors for infections in different settings. Most of the Georgian population is likely infected by *H. pylori*,[12,13] and testing and specific treatment for the infection is not routinely practiced in ROG. Therefore, it is likely that a substantial burden of *H. pylori*-associated morbidity and mortality in the Georgian population could be prevented by the introduction of routine diagnostic testing and specific treatment of symptomatic patients. Due to the limited sensitivity of ELISA and CLO, we recommend using histology or UBT in ROG. Antimicrobial therapy against *H. pylori* is highly effective and serious risks associated with testing and treatment are minimal. There is a need for more research to identify risk factors for acquiring the initial *H. pylori* infection so that primary prevention strategies can be designed.

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References

1. Marshall B, Warren J. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;**1**:1311–5.
2. Suerbaum S, Michetti P. *Helicobacter pylori* infections. *N Engl J Med* 2002; **347**:1175–86.
3. Chey W, Wong B. Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. *Am J Gastroenterol* 2007; **102**:1808–25.
4. Lacy BE, Rosemore J. *Helicobacter pylori*: ulcers and more: the beginning of an era. *J Nutr* 2001;**131**. 2789S–2793S.
5. Makola D, Peura D, Crowe S. *Helicobacter pylori* infection and related gastrointestinal diseases. *J Clin Gastroenterol* 2007;**41**:548–58.
6. Jones NL, Sherman PM. *Helicobacter pylori* infection in children. *Curr Opin Pediatr* 1998;**10**:19–23.
7. Fiedorek SC, Malaty HM, Evans DL, Pumphrey CL, Casteel HB, Evans Jr DJ, et al. Factors influencing the epidemiology of *Helicobacter pylori* infection in children. *Pediatrics* 1991;**88**:578–82.
8. Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, et al. Childhood living conditions and *Helicobacter pylori* seropositivity in adult life. *Lancet* 1992;**339**:896–7.
9. Graham DY, Adam E, Reddy GT, Agarwal JP, Agarwal R, Evans Jr DJ, et al. Seroepidemiology of *Helicobacter pylori* infection in India. Comparison of developing and developed countries. *Dig Dis Sci* 1991;**36**:1084–8.
10. Guarner J, Kalach N, Elitsur Y, Koletzko S. Helicobacter pylori diagnostic tests in children: review of the literature from 1999 to 2009. *Eur J Pediatr* 2010;**169**: 15–25.
11. Barthel JS, Everett ED. Diagnosis of *Campylobacter pylori* infections: the "gold standard" and the alternatives. *Rev Infect Dis* 1990;**12**(Suppl 1):S107–14.
12. Kretsinger K, Sobel J, Tarkhashvili N, Chakvetadze N, Moistrafishvili M, Sikharulidze M, et al. *Helicobacter pylori*, Republic of Georgia. *Emerg Infect Dis* 2005;**11**:780–1.
13. Olivares A, Buadze M, Kutubidze T, Lobjanidze M, Labauri L, Kutubidze. et al. Prevalence of *Helicobacter pylori* in Georgian patients with dyspepsia. *Helicobacter* 2006;**11**:81–5.
14. Tarkhashvili N, Beriashvili R, Chakvetadze N, Moistsrapishvili M, Chokheli M, Sikharulidze M, et al. *Helicobacter pylori* infection in patients undergoing upper endoscopy, Republic of Georgia. *Emerg Infect Dis* 2009;**15**:504–5.
15. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the updated Sydney system. *Am J Surg Pathol* 1996;**20**:1161–81.
16. Khanna B, Cutler A, Israel NR, Perry M, Lastovica A, Fields PI, et al. Use caution with serologic testing for *Helicobacter pylori* infection in children. *J Infect Dis* 1998;**178**:460–5.
17. Parkinson AJ, Gold BD, Bulkow L, Wainwright RB, Swaminathan B, Khanna B, et al. High prevalence of *Helicobacter pylori* in the Alaska Native population and association with low serum ferritin levels in young adults. *Clin Diagn Lab Immunol* 2000;**7**:885–8.
18. Hisada M, Lee MG, Hanchard B, Owens M, Song Q, van Doorn LJ, et al. Characteristics of *Helicobacter pylori* infection in Jamaican adults with gastrointestinal symptoms. *J Clin Microbiol* 2001;**39**:212–6.
19. Sharapov U, Schmitz A, Krueger A, Fagan R, Chakvetadze N, Barzilay E, et al. High prevalence of *Helicobacter pylori* infection, and increased proportion of seropositive children after age 4 years in school children in two cities in the Republic of Georgia. Abstract 934. Abstracts of the 48th Annual Meeting of the Infectious Diseases Society of America, Vancouver, October 21–24, 2010.
20. Zhang C, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. *Helicobacter pylori* infection, glandular atrophy and intestinal metaplasia in superficial gastritis, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. *World J Gastroenterol* 2005;**11**:791–6.
21. Bardhan PK. Epidemiological features of *Helicobacter pylori* infection in developing countries. *Clin Infect Dis* 1997;**25**:973–8.
22. Harbour S, Sutton P. Immunogenicity and pathogenicity of Helicobacter infections of veterinary animals. *Vet Immunol Immunopathol* 2008;**122**:191–203.
23. Fox JG. Non-human reservoirs of *Helicobacter pylori*. *Aliment Pharmacol Ther* 1995;**9**(Suppl 2):93–103.
24. O'Rourke JL, Grehan M, Lee A. Non-pylori *Helicobacter* species in humans. *Gut* 2001;**49**:601–6.
25. Kalach N, Mention K, Guimber D, Michaud L, Spyckerelle C, Gottrand F. *Helicobacter pylori* infection is not associated with specific symptoms in non-ulcer-dyspeptic children. *Pediatrics* 2005;**115**:17–21.
26. Motta CR, Cunha MP, Queiroz DM, Cruz FW, Guerra EJ, Mota RM, et al. Gastric precancerous lesions and *Helicobacter pylori* infection in relatives of gastric cancer patients from northeastern Brazil. *Digestion* 2008;**78**:3–8.
27. El-Omar EM, Oien K, Murray LS, El-Nujumi A, Wirz A, Gillen D, et al. Increased prevalence of precancerous changes in relatives of gastric cancer patients: critical role of *H. pylori*. *Gastroenterology* 2000;**118**:22–30.
28. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China. *JAMA* 2004;**291**:187–94.
29. Genta RM, Gürer IE, Graham DY, Krishnan B, Segura AM, Gutierrez O, et al. Adherence of *Helicobacter pylori* to areas of incomplete intestinal metaplasia in the gastric mucosa. *Gastroenterology* 1996;**111**:1206–11.
30. Bravo LE, Realpe JL, Campo C, Mera R, Correa P. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for *Helicobacter pylori* infection. *Am J Gastroenterol* 1999;**94**:2380–3.